Viscosities of Aqueous Solutions of Human Serum Albumin in the Presence of Two Anionic Penicillins at pH 7.4

Silvia Barbosa,* Pablo Taboada, and Víctor Mosquera

Grupo de Física de Coloides y Polímeros, Departamento de Física da Materia Condensada, Facultade de Física, Universidade de Santiago de Compostela, 15782, Santiago de Compostela, Spain

Relative viscosity values for cloxacillin and dicloxacillin penicillins in an aqueous solution of pH 7.4 in the presence of human serum albumin at concentrations of 4 g L^{-1} and 20 g L^{-1} have been derived from measurements made with a microviscometer based on the "rolling ball" principle. The viscosities have been measured in the range of the temperatures (20 to 50) °C.

1. Introduction

Interactions between surfactant molecules and natural polymers have been extensively reviewed.[1−3] These studies have shown that complexes can be formed between proteins and surfactants in the bulk phase. Two types of protein/surfactant interactions have been proposed: (a) specific binding via electrostatic attraction and (b) cooperative association of the surfactant to the protein via hydrophobic affinity. These interactions can give rise to important changes to the physicochemical properties of biopolymer systems and play an important role in biological and pharmacological applications. Human serum albumin (HSA) is used as a model protein for studying the interaction between proteins and different surface substrates.[4,5] HSA is a globular protein that consists of 585 amino acids in a single polypeptide chain with a molar mass of 66 411 g mol^{-1}, and it constitutes approximately half of the total blood protein, acting as carrier for fatty acids and several amphiphiles from bloodstream to tissues and hence is an appropriate choice of protein for studying the interaction with amphiphilic drugs.[6−7]

In this work, we report determinations of viscosities for HSA−penicillin complexes with the HSA concentration, (4 and 20) g L^{-1}, and at the temperatures of (20, 30, 40, and 50) °C and pH 7.4. The penicillins chosen were cloxacillin and dicloxacillin, two structurally related anionic amphiphilic penicillins, which differ only in an additional chlorine atom on the phenyl ring of dicloxacillin (see Figure 1). The globular protein HSA is negatively charged at pH 7.4, and therefore only hydrophobic interactions are established between protein and the penicillins. The measurements were done above the isoelectric point of the protein (4.9) because the change in the pH alters the charge distributions of the protein molecule and hence the stability and tendency for surface adsorption.[8]

2. Experimental Section

Materials. HSA (70024-90-7), sodium cloxacillin monohydrate (5- methyl-3-(*o*-chlorophenyl)-4-isoxazolyl) and sodium dicloxacillin monohydrate (3-(2,6-dichlorophenyl)-5-methyl-α-isoxazolyl) with at least 98.5% purity were obtained from Sigma Chemical Company. The molecular weights of cloxacillin and dicloxacillin are 475.9 g mol^{-1} and 510.3 g mol^{-1}, respectively. Experiments were carried out using phosphate buffered saline tablets for pH 7.4 with ionic strength of 0.188 M. The solutions were weighted on a Scaltec balance with a precision of ±0.00002 g. Water was double distilled and deionized before use.

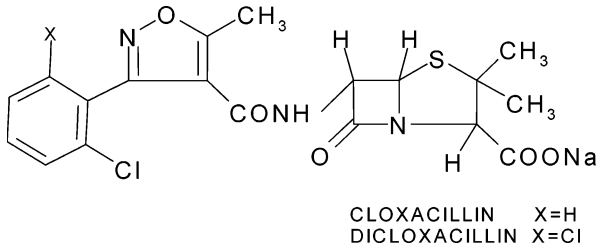

Figure 1. Molecular structure of the penicillins.

Adsorption of Cloxacillin and Dicloxacillin onto HSA. Aliquots of 2 mL of 8 g L^{-1} (for 4 g L^{-1} of HSA) and 40 g L^{-1} (for 20 g L^{-1} of HSA) of HSA were added to equal volumes of aqueous solutions of penicillin of known concentration to yield final solutions that the concentrations of HSA were 4 g L^{-1} and 20 g L^{-1}, respectively. The solutions were maintained at 20 °C for the necessary time until equilibrium was achieved.

Viscosity. Measurements were made with an Anton Paar Automated Microviscometer (AMVn). The AMVn is based on the "rolling-ball" principle. The samples of buffer or buffer−HSA−penicillin solutions were introduced into a glass capillary with a diameter of 1.6 mm (recommended viscosity measuring range 0.3 to 10 mPa s) in which the steel ball rolls. The viscous properties of the test fluid can be determined by measuring the rolling time of the steel ball. The measurements were made in the range of temperatures from 20 °C to 50 °C. The temperature was maintained by the Peltier effect with a resolution of ±0.01 °C. The precision in the measurements was ±0.00004 mol kg^{-1} for the concentration and for the rolling time of the ball through the solution, t, of ±0.002 s. For the relative viscosity, η_{rel}, the uncertainty is 0.10%.

The rolling times of the steel ball in the buffer were (22.746 ± 0.002) s at 20 °C, (18.170 ± 0.002) s at 30 °C, (14.977 ± 0.003) s at 40 °C, and (12.681 ± 0.005) s at 50 °C in the presence of HSA at a concentration of 4 g L^{-1} at pH 7.4 and (24.272 ± 0.001) s at 20 °C, (19.385 ± 0.003) s at 30 °C, (15.950 ± 0.002) s at 40 °C, and (13.466 ± 0.003)

* To whom correspondence may be addressed. E-mail: fmsilvia@usc.es.

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Table 1. Concentration, c, Rolling Time of the Ball through the Solution, τ, and Relative Viscosity, η_{rel}, for Cloxacillin in the Presence of HSA at a Concentration of 4 and 2 g L^{-1} at pH 7.4 and at Different Temperatures

	t = 20 °C		t = 30 °C		t = 40 °C		t = 50 °C	
c/(mol·kg^{-1})	τ/s	η_{rel}	τ/s	η_{rel}	τ/s	η_{rel}	τ/s	η_{rel}
Cloxacillin + 4 g L^{-1} HSA								
0.000 48	22.765	1.000 83	18.192	1.001 21	14.988	1.000 73	12.685	1.000 31
0.001 06	22.807	1.002 68	18.227	1.003 14	15.004	1.001 80	12.693	1.000 95
0.002 08	22.834	1.003 87	18.233	1.003 47	15.020	1.002 87	12.704	1.001 81
0.005 13	22.926	1.007 91	18.306	1.007 48	15.077	1.006 68	12.750	1.005 44
0.010 12	23.061	1.013 85	18.413	1.013 37	15.161	1.012 28	12.819	1.010 88
0.019 87	23.412	1.029 28	18.684	1.028 29	15.362	1.025 71	12.971	1.022 88
0.039 91	24.153	1.061 86	19.220	1.057 79	15.778	1.053 48	13.305	1.049 27
0.099 88	26.714	1.174 45	21.139	1.163 40	17.271	1.153 17	14.496	1.143 12
Cloxacillin + 20 g L^{-1} HSA								
0.000 52	24.377	1.004 33	19.431	1.002 37	15.991	1.002 57	13.497	1.002 30
0.002 33	24.396	1.005 11	19.483	1.005 05	16.016	1.004 14	13.508	1.003 12
0.009 73	24.712	1.018 13	19.730	1.017 80	16.235	1.017 87	13.696	1.017 08
0.024 06	25.213	1.038 77	20.106	1.037 19	16.521	1.035 80	13.912	1.033 12
0.039 36	26.344	1.085 37	20.919	1.079 13	17.137	1.074 42	14.410	1.070 10
0.098 95	29.721	1.224 50	23.368	1.205 47	19.042	1.193 85	16.049	1.191 82
0.196 96	37.041	1.526 08	29.351	1.514 11	24.025	1.506 27	20.130	1.494 88
0.297 95	24.377	1.004 33	19.431	1.002 37	15.991	1.002 57	13.497	1.002 30
0.330 14	57.015	2.349 00	44.372	2.288 99	35.485	2.224 76	29.151	2.164 78

Table 2. Concentration, c, Rolling Time of the Ball through the Solution, τ, and Relative Viscosity, η_{rel}, for Dicloxacillin in the Presence of HSA at a Concentration of 4 and 20 g L^{-1} at pH 7.4 and at Different Temperatures

	t = 20 °C		t = 30 °C		t = 40 °C		t = 50 °C	
c/(mol·kg^{-1})	τ/s	η_{rel}	τ/s	η_{rel}	τ/s	η_{rel}	τ/s	η_{rel}
Dicloxacillin + 4 g L^{-1} HSA								
0.000 53	22.748	1.000 09	18.175	1.000 27	14.979	1.000 13	12.683	1.000 16
0.000 98	22.758	1.000 53	18.186	1.000 88	14.981	1.000 27	12.686	1.000 39
0.002 06	22.765	1.000 83	18.194	1.001 32	14.984	1.000 47	12.688	1.000 55
0.005 12	22.874	1.005 63	18.256	1.004 73	15.046	1.004 60	12.723	1.003 31
0.010 10	23.038	1.012 84	18.389	1.012 05	15.138	1.010 75	12.792	1.008 75
0.019 88	23.374	1.027 61	18.644	1.026 09	15.333	1.023 77	12.944	1.020 74
0.038 51	24.204	1.064 10	19.257	1.059 82	15.791	1.054 35	13.313	1.049 84
0.099 88	27.395	1.204 39	21.714	1.195 05	17.751	1.185 21	14.869	1.172 54
Dicloxacillin + 20 g L^{-1} HSA								
0.000 78	24.305	1.001 36	19.395	1.000 52	15.958	1.000 50	13.476	1.000 74
0.001 35	24.299	1.001 11	19.399	1.000 72	15.969	1.001 19	13.485	1.001 41
0.002 61	24.398	1.005 19	19.458	1.003 77	16.006	1.003 51	13.510	1.003 27
0.010 20	24.760	1.020 11	19.745	1.018 57	16.241	1.018 24	13.717	1.018 64
0.025 10	25.383	1.045 77	20.200	1.042 04	16.593	1.040 31	13.978	1.038 02
0.049 48	27.344	1.126 57	21.657	1.117 20	17.720	1.110 97	14.934	1.109 01
0.098 73	31.957	1.316 62	25.306	1.305 44	20.705	1.298 11	17.352	1.288 58

s at 50 °C in the presence of HSA at a concentration of 20 g L^{-1} at pH 7.4.

3. Results and Discussion

Experimental results are given in Table 1 and Table 2 for cloxacillin and dicloxacillin at pH 7.4 and at different HSA concentrations, respectively. The relative viscosity was calculated using the equation

$$\eta_{rel} = \frac{\tau}{\tau_0} \quad (1)$$

where τ/s is the rolling time of the ball through the solution and τ_0/s is the rolling time of the ball through the buffer with the protein.

In Figures 2 and 3, the increase of the relative viscosity with the concentration of cloxacillin and dicloxacillin can be observed, respectively, together with the decrease of this magnitude when the temperature increases for both HSA concentrations. Two characteristic regions are identified in both figures: (a) The first region is a plateau where viscosity remains almost constant up to concentrations close to 0.01 mol kg^{-1}. In this region, the viscosity is almost unaffected by the presence of penicillin. (b) The second region is where the viscosity steeply rises as a consequence

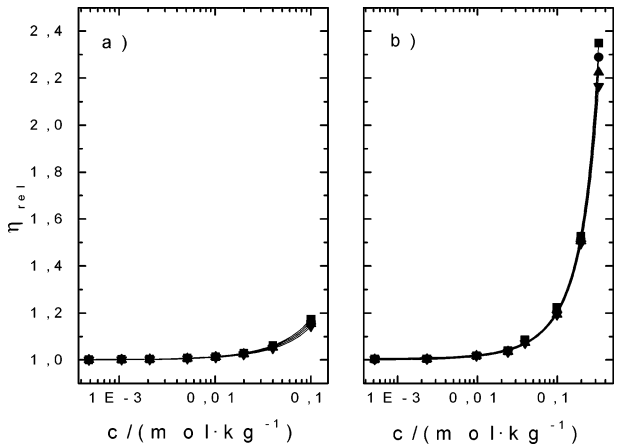

Figure 2. Specific viscosity, η_{rel}, vs concentration, c, of cloxacillin at pH 7.4 in the presence of HSA at a concentration of (a) 4 g L^{-1} and (b) 20 g L^{-1} at different temperatures. ■, 20 °C; ●, 30 °C; ▲, 40 °C; ▼, 50 °C.

of an increase in number and transformation in the shape of the protein molecules as has been demonstrated by dynamic light-scattering measurements.[9] It has been proposed by isothermal titration calorimetry that the binding process of penicillins onto HSA can be described as a two

Table 3. Coefficients A_1, A_2, and A_3 of Equation 2 for Dicloxacillin and Cloxacillin in the Presence of HSA at Concentrations 4 and 20 g L^{-1} at pH 7.4 and at Different Temperatures

	4 g L^{-1} HSA			20 g L^{-1} HSA		
	A_1	A_2	A_3	A_1	A_2	A_3
			Dicloxacillin			
t = 20 °C	0.767 ± 0.030	0.231 ± 0.030	0.157 ± 0.015	0.791 ± 0.048	0.207 ± 0.046	0.106 ± 0.015
t = 30 °C	0.805 ± 0.026	0.193 ± 0.022	0.142 ± 0.012	0.830 ± 0.033	0.168 ± 0.031	0.095 ± 0.011
t = 40 °C	0.847 ± 0.013	0.152 ± 0.013	0.124 ± 0.007	0.855 ± 0.024	0.143 ± 0023	0.088 ± 0.008
t = 50 °C	0.871 ± 0.017	0.127 ± 0.016	0.115 ± 0.010	0.855 ± 0.031	0.143 ± 0.030	0.083 ± 0.011
			Cloxacillin			
t = 20 °C	0.668 ± 0.035	0.332 ± 0.035	0.237 ± 0.021	0.693 ± 0.032	0.312 ± 0.029	0.198 ± 0.009
t = 30 °C	0.692 ± 0.032	0.309 ± 0.032	0.236 ± 0.020	0.689 ± 0.017	0.314 ± 0.015	0.203 ± 0.005
t = 40 °C	0.724 ± 0.014	0.276 ± 0.014	0.226 ± 0.009	0.665 ± 0.013	0.336 ± 0.012	0.215 ± 0.004
t = 50 °C	0.760 ± 0.001	0.239 ± 0.010	0.212 ± 0.007	0.634 ± 0.015	0.364 ± 0.014	0.230 ± 0.047

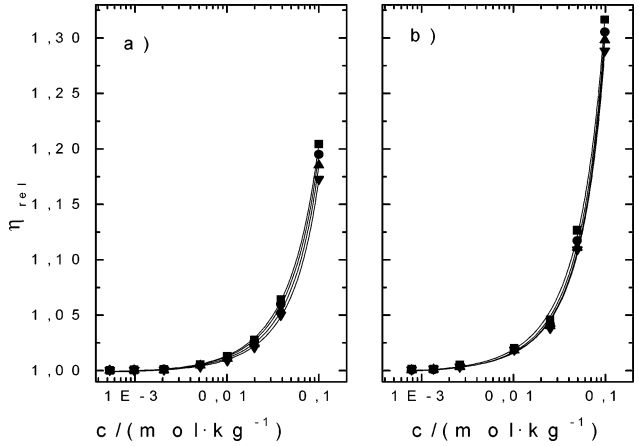

Figure 3. Specific viscosity, η_{rel}, vs concentration, c, for dicloxacillin at pH 7.4 in the presence of HSA at a concentration of (a) 4 g L^{-1} and (b) 20 g L^{-1} at different temperatures. ■, 20 °C; ●, 30 °C; ▲, 40 °C; ▼, 50 °C.

binding site model, in which the first site would bind just one penicillin molecule and the second site the rest of the adsorbed penicillin molecules (about 15−25 penicillin molecules depending on the compound).[10−11]

The variation of the relative viscosity with the penicillin concentration, c, can be described by the exponential equation

$$\eta_{rel} = A_1 + A_2\, e^{c/A_3} \qquad (2)$$

with values of the coefficients A_1, A_2, and A_3 given in Table 3.

The effect of protein concentration can be observed in the Figures 2 and 3. At 20 g L^{-1} of protein, the plateau extends to a higher penicillin concentration as a consequence of the existence of more protein molecules available for binding and, thus, delays the configuration changes of the protein. The differences between the relative viscosities of the protein at the concentration of 20 g L^{-1} and in the presence of some of the penicillins in the temperature range of the present study are minimum.

Literature Cited

(1) Tanford, C. *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*; Willey Interscience: New York, 1980.
(2) Ananthapadmanabham, K. P. *Interactions of Surfactants with Polymers and Proteins*; Goddard, E. D., Ananthapadmanabhan D. P., Eds.; CRC Press, Inc.: London, 1993.
(3) Kwak, J. C. T. *Polymer-Surfactant Systems; Surfactant Science Series*; Kwak, J. C. T., Ed.; Marcel Dekker: New York, 1998; Vol. 77.
(4) Peters, T. J. *All About Albumin Biochemistry, Genetics, and Medical Applications*; Academic Press: San Diego, CA, 1996.
(5) Taboada, P.; Mosquera, V.; Ruso, J. M.; Sarmiento, F.; Jones, M. N. Interaction between Penicillins and Human Serum Albumin: A Thermodynamic Study of Micellar-Like Clusters on a Protein. *Langmuir* **2000**, *16*, 934−938.
(6) Taboada, P.; Mosquera, V.; Ruso, J. M.; Sarmiento, F.; Jones, M. N. Interaction between Penicillins and Human Serum Albumin: A ξ-Potential Study. *Langmuir* **2000**, *16*, 6795−6800.
(7) Barbosa, S.; Leis, D.; Taboada, P.; Atwood, D.; Mosquera, V. A Study of the Adsorption of the Amphiphilic Penicillins Cloxacillin and Dicloxacill onto Human Serum Albumin using Surface Tension Isotherms. *Mol. Phys.* **2002**, *21*, 3367−3374.
(8) Lu, J. R.; Su, T. J. and Penfold, J. Adsorption of Serum Albumins at the Air/Water Interface. *Langmuir* **1999**, *15*, 6975−6983.
(9) Ruso, J. M.; Attwood, D.; García, M.; Taboada, P.; Varela, L. M.; Mosquera, V. A Study of the Interaction of the Amphiphilic Penicillins Cloxacillin and Dicloxacillin whith Human Serum Albumin in Aqueous Solution. *Langmuir* **2001**, *17*, 5189−5195.
(10) Landau, M. A.; Markovich, M. N.; Piruzyan, L. A. Studies of the Thermodinamics and Nature of Interaction between Serum Albumin and Penicillins. *Biochim. Biophys. Acta* **1977**, *493*, 1−9.
(11) Barbosa, S.; Taboada, P.; Mosquera, V. Protein−Ligand Interactions: Volumetric and Compressibility Characterization of the Binding of Two Anionic Penicillins to Human Serum Albumin. *Langmuir* **2003**, *19*, 1446−1448.

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